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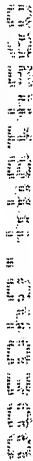


Figure 2 - Process for Production of hGH from Ala-hGH

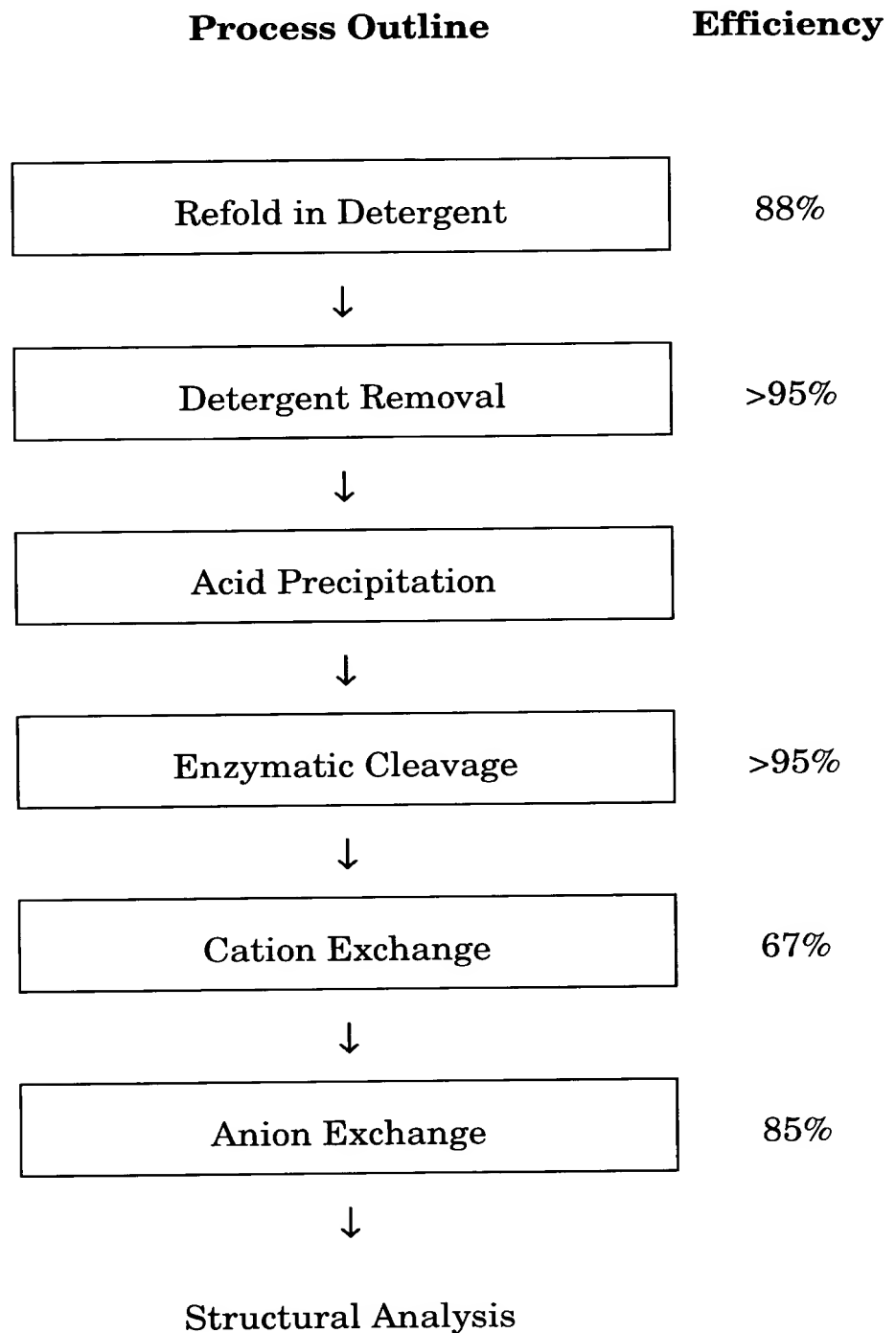
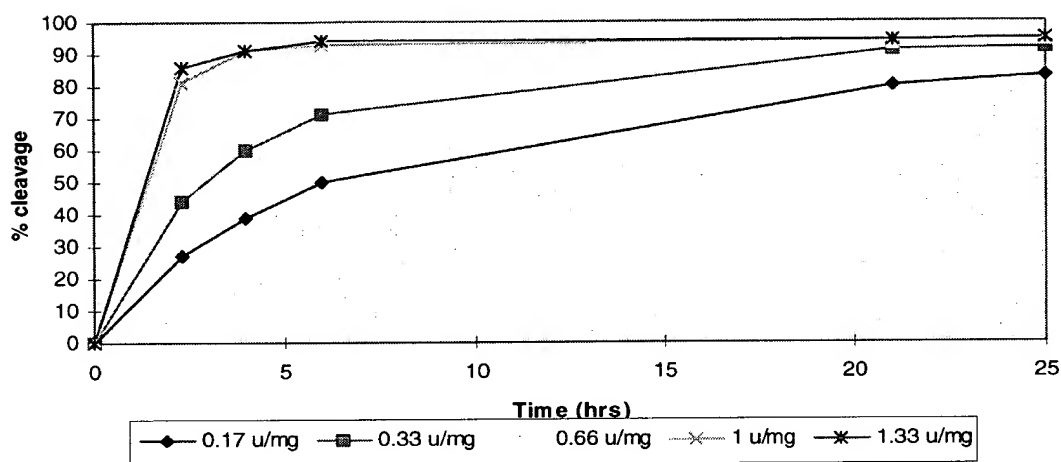
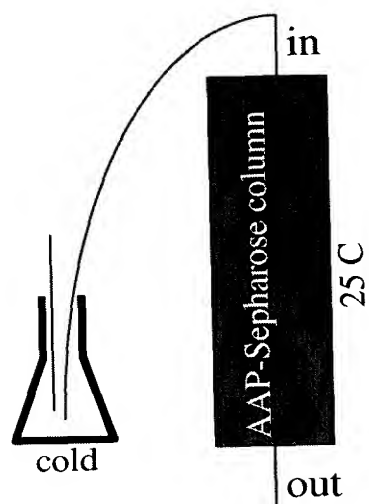


Figure 3 - Kinetics of removal of Ala from Ala-hGH by AAP

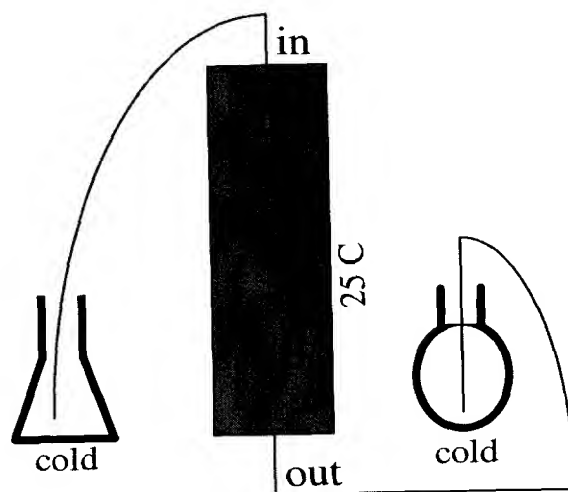


Studies of enzymatic removal of alanine from Ala-hGH: [Ala-hGH] 3 mg/ml; [AAP] 0.5, 1.0, 2.0, 3.0, and 4.0 units/ml; volume 6 ml. Cleavage efficiencies were determined by ES/MS. All reactions were performed at room temperature.

Figure 4 - Enzymatic cleavage process options - Column mode



Re-circulation mode
192 ml/hr; 10 passes/24 hr
Residence time= 180 min



Flow-through mode
19.8 ml/hr; 1 pass
Residence time= 175 min

Figure 5 - Batch mode cleavage of Ala-hGH

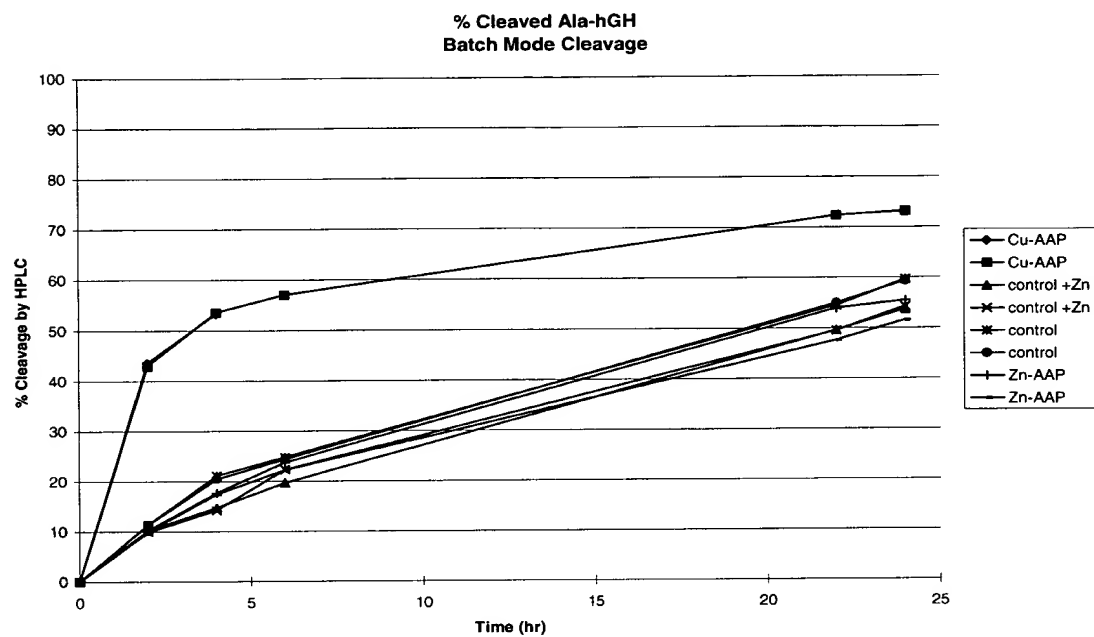


Figure 6 - Recirculation mode cleavage of Ala-hGH

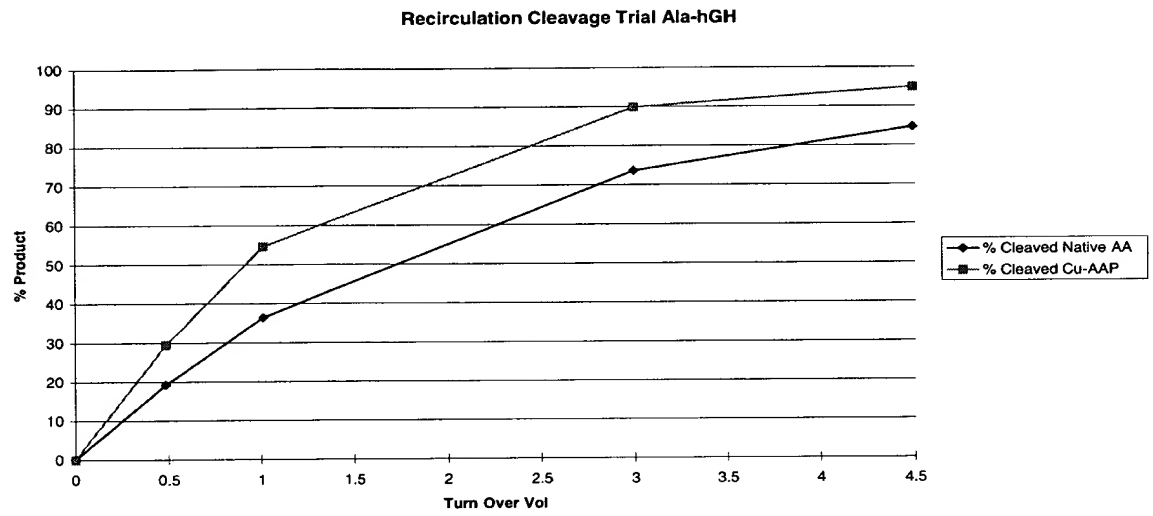
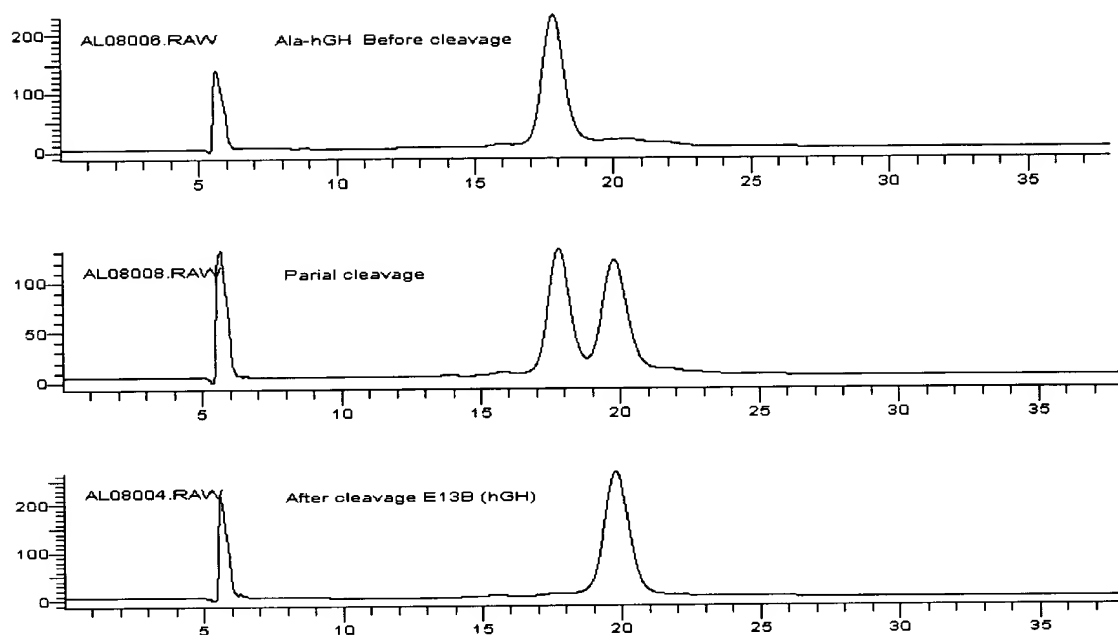


Figure 7 - Cleavage efficiency analyzed by HPLC



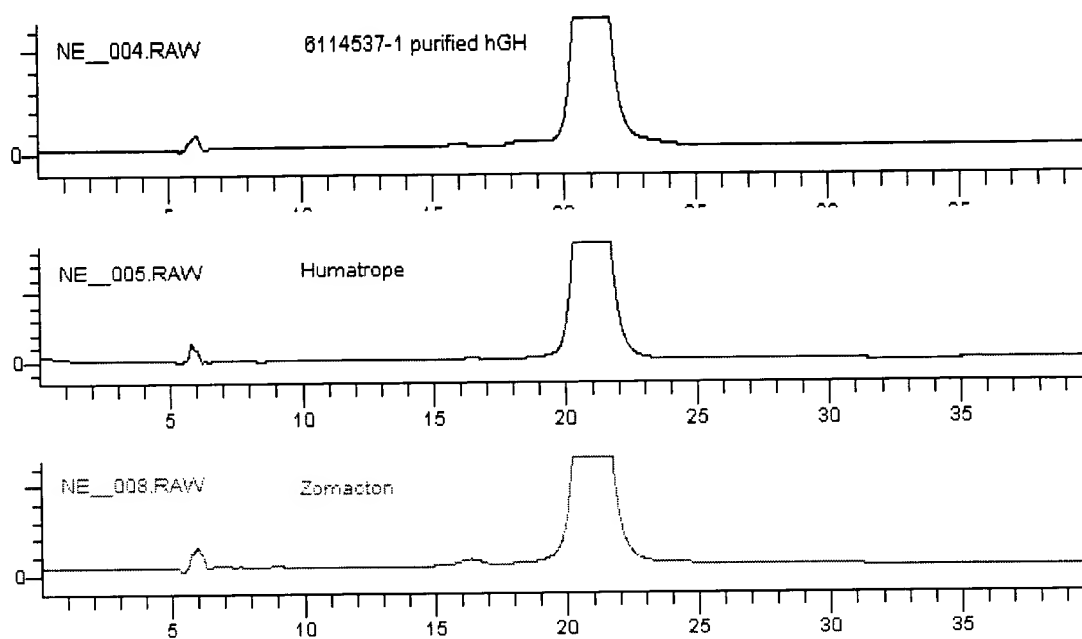
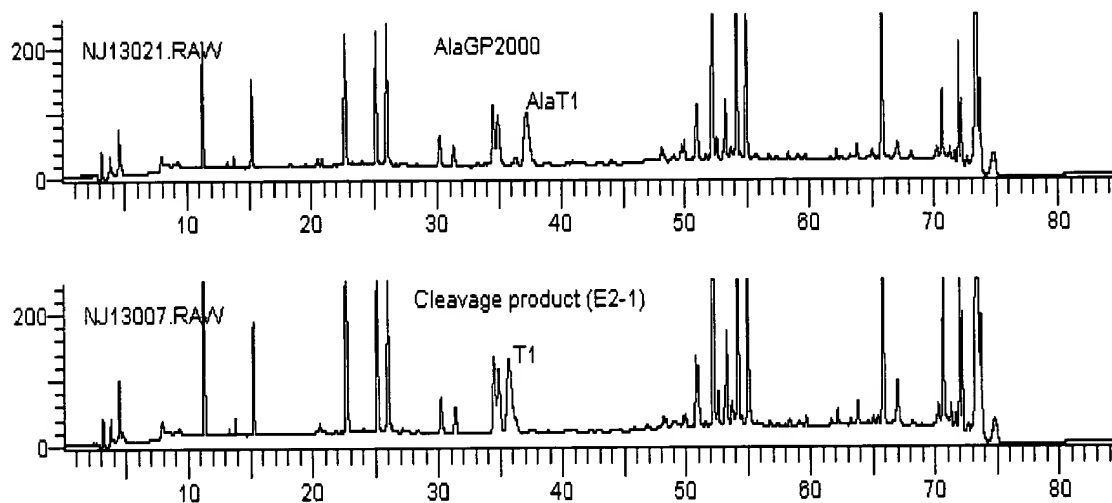
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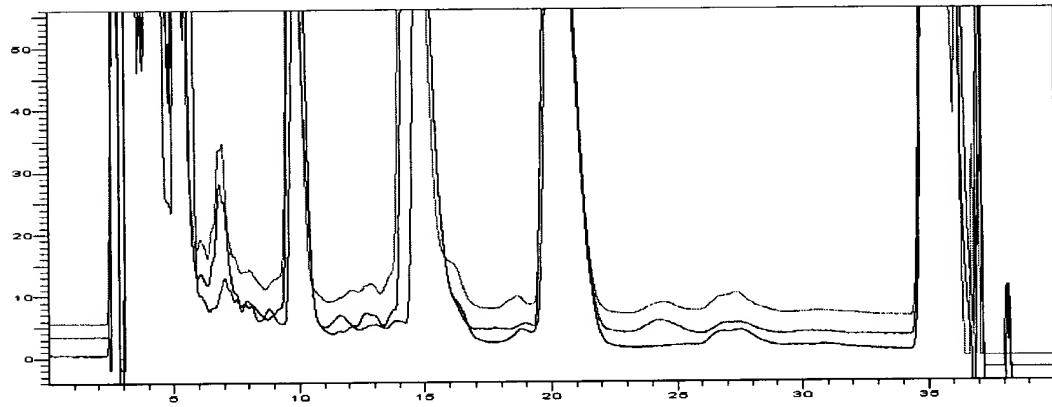
Figure 9 - Products of Ala-hGH treated with AAP analyzed by tryptic mapping



Ala-T1: N-terminal peptide from Ala-hGH (top) (AFPTIPLSR)

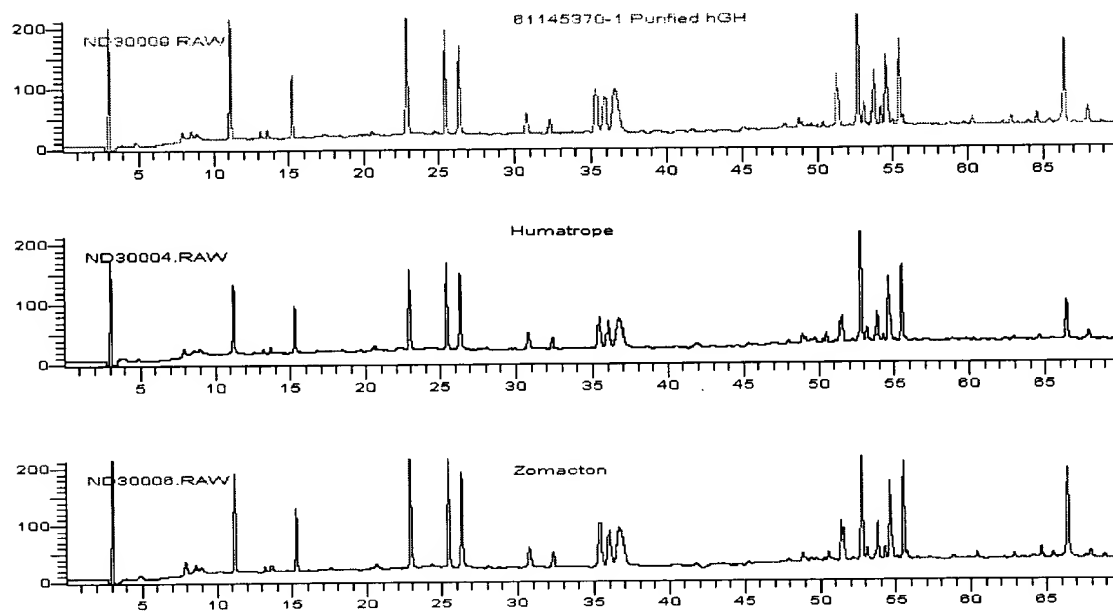
T1: N-terminal peptide from hGH (bottom) (FPTIPLSR)

Figure 10 - Quantitative analysis of residual Ala-hGH in hGH by tryptic digest



Baseline resolution of Ala-T1 and T1 peptides allows quantification of residual uncleaved Ala-hGH.

Figure 11 - Product comparison by tryptic mapping



試料名	試料番号	試料重量 (g)	試料体積 (cm ³)	試料密度 (g/cm ³)	試料組成 (wt%)	試料特性
試料 A	試料 A-1	10.0	1.0	10.0	100.0	試料 A-1
試料 B	試料 B-1	10.0	1.0	10.0	100.0	試料 B-1
試料 C	試料 C-1	10.0	1.0	10.0	100.0	試料 C-1
試料 D	試料 D-1	10.0	1.0	10.0	100.0	試料 D-1
試料 E	試料 E-1	10.0	1.0	10.0	100.0	試料 E-1
試料 F	試料 F-1	10.0	1.0	10.0	100.0	試料 F-1
試料 G	試料 G-1	10.0	1.0	10.0	100.0	試料 G-1
試料 H	試料 H-1	10.0	1.0	10.0	100.0	試料 H-1
試料 I	試料 I-1	10.0	1.0	10.0	100.0	試料 I-1
試料 J	試料 J-1	10.0	1.0	10.0	100.0	試料 J-1
試料 K	試料 K-1	10.0	1.0	10.0	100.0	試料 K-1
試料 L	試料 L-1	10.0	1.0	10.0	100.0	試料 L-1
試料 M	試料 M-1	10.0	1.0	10.0	100.0	試料 M-1
試料 N	試料 N-1	10.0	1.0	10.0	100.0	試料 N-1
試料 O	試料 O-1	10.0	1.0	10.0	100.0	試料 O-1
試料 P	試料 P-1	10.0	1.0	10.0	100.0	試料 P-1
試料 Q	試料 Q-1	10.0	1.0	10.0	100.0	試料 Q-1
試料 R	試料 R-1	10.0	1.0	10.0	100.0	試料 R-1
試料 S	試料 S-1	10.0	1.0	10.0	100.0	試料 S-1
試料 T	試料 T-1	10.0	1.0	10.0	100.0	試料 T-1
試料 U	試料 U-1	10.0	1.0	10.0	100.0	試料 U-1
試料 V	試料 V-1	10.0	1.0	10.0	100.0	試料 V-1
試料 W	試料 W-1	10.0	1.0	10.0	100.0	試料 W-1
試料 X	試料 X-1	10.0	1.0	10.0	100.0	試料 X-1
試料 Y	試料 Y-1	10.0	1.0	10.0	100.0	試料 Y-1
試料 Z	試料 Z-1	10.0	1.0	10.0	100.0	試料 Z-1

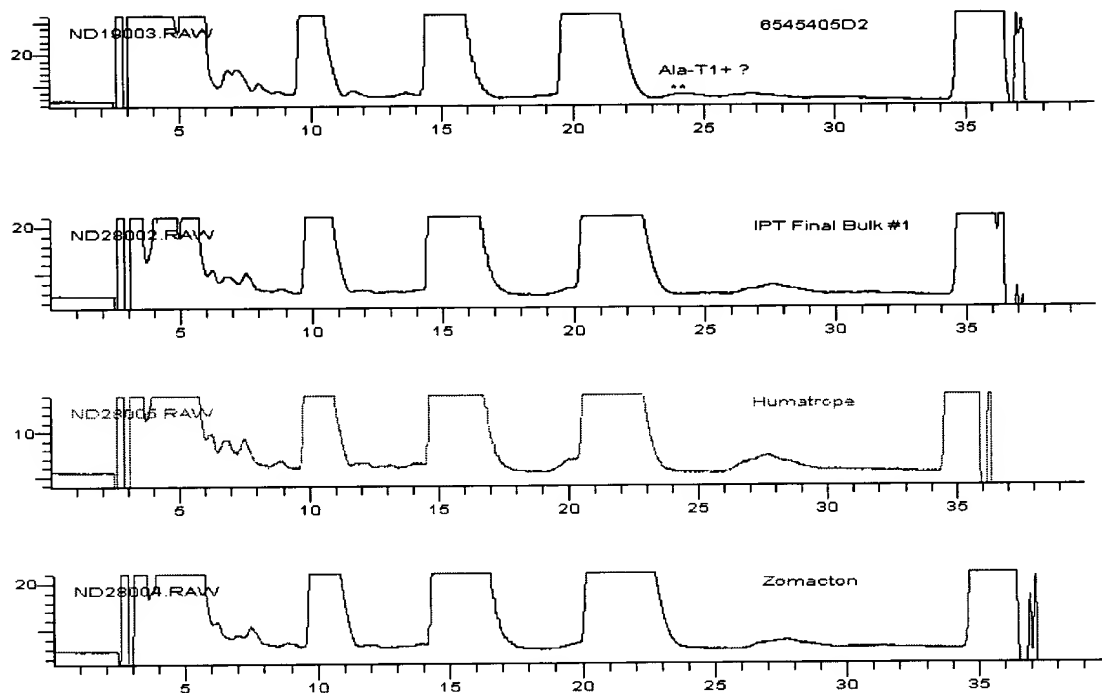
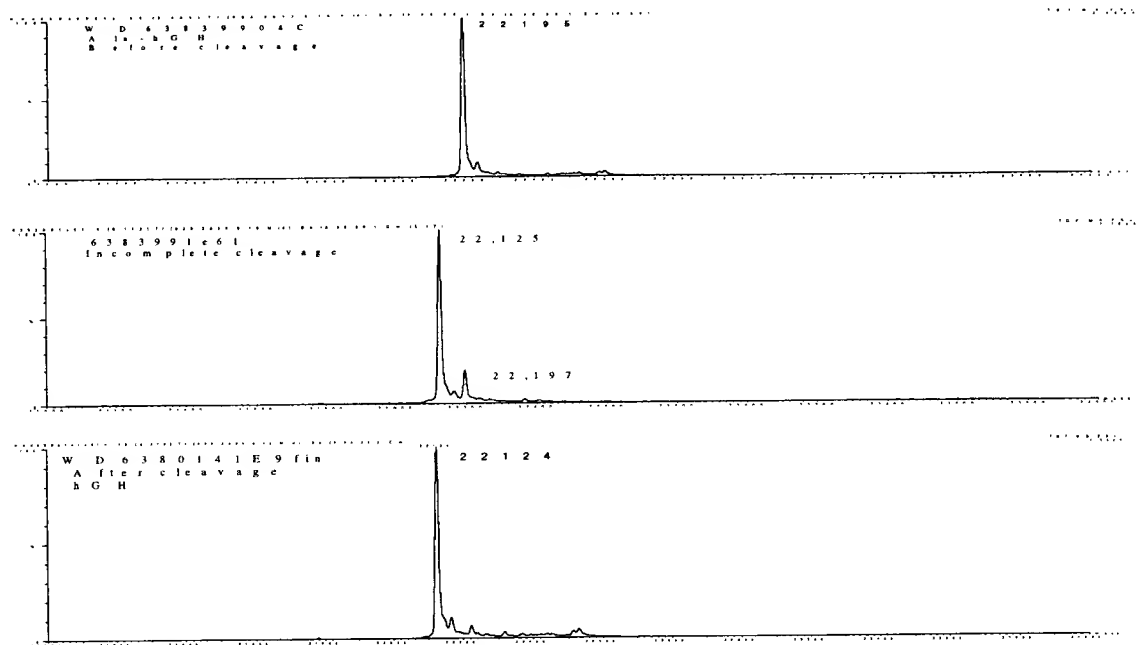


Figure 13 - Cleavage efficiency analyzed by ES/MS



100
80
60
40
20
0

Figure 14 - Product analysis by RP-HPLC, SE-HPLC, and ES/MS

PRODUCT ANALYSIS

Top: RP-HPLC (Vydac C18) using 65-85%/20 min acetonitrile gradient in 1% TFA

Center: SE-HPLC (BioRad Bio-Sil SEC 250) using 60% acetonitrile/80 mM TFA isocratic

Bottom: ES/MS: Ala-hGH (22,198); hGH (22,125)

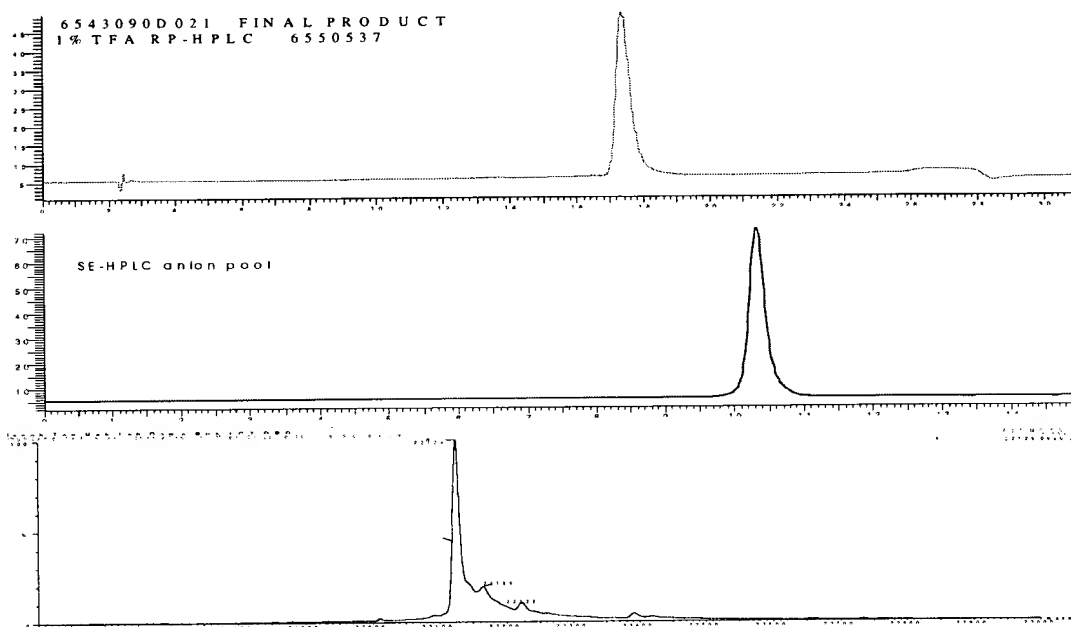


Figure 15 - Product analyzed by N-terminal sequencing

In cycle 1 (left), Phe (18.3 min) is the only significant residue detected; Proline (14.2 min) is the expected residue for cycle 2 (right). The amount of alanine (*, is negligible in both cycles indicating no Ala-hGH in the product.

